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EXCESS POTASSIUM INDUCES LARVAL METAMORPHOSIS IN FOUR MARINE INVERTEBRATE SPECIES

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Abstract

An increase in the concentration of K^+ in defined seawater medium induces settlement and metamorphosis in larvae of the marine molluscs *Phestilla sibogae, Haliotis rufescens,* and *Astraea undosa,* and in larvae of the marine annelid *Phragmatopoma californica.* The effect is dose-dependent, optimal at approximately double the normal concentration of K^+ in seawater, and specific for the K^+ ion. The ability of K^+ to directly influence cell membrane potential is proposed as an explanation for its broad effectiveness as a metamorphic inducer for larvae that recruit to different habitats. Depolarization of externally accessible, excitable cells thus is suggested to be a mechanism common to the induction of settlement and metamorphosis of a number of species. For *Phestilla* and *Haliotis,* the inductive effect of excess K^+ is additive with that of the substratum-derived inducers or analogs. The sensitivity of induction by K^+ to external tetraethylammonium (TEA, a K^+-channel blocker) reported previously for *Haliotis* (Baloun and Morse, 1984) is not present in *Phestilla* or *Phragmatopoma.* Results presented here indicate that the addition of excess K^+ may provide a widely useful technique for inducing metamorphosis, and for analyzing the mechanisms which govern this process, in other marine invertebrate larvae.

Introduction

Specific environmental stimuli are required for the initiation of metamorphosis by larvae of many marine invertebrates. In the plankton, these larvae typically develop competence to respond to metamorphic inducers, but do not proceed through metamorphosis in the absence of an appropriate stimulus. Often the stimulus is derived from a substratum capable of providing the newly settled juveniles with a source of nutrition and refuge; perception of this stimulus by larvae can involve chemical, tactile, or visual modalities (reviews by Crisp, 1974; and Hadfield, 1978). The sensory basis of induction suggests that the nervous system is involved in the initiation of metamorphosis (Hadfield, 1978; Burke, 1983a, b). In several species, substratum-associated morphogenetic chemical cues have been found to be neurotransmitter-mimetic substances (Morse, 1986); in these and other cases, exogenous neurotransmitters can elicit metamorphic responses similar to those induced by the natural cues, thus further implicating neuronal receptors in the initial processes (Bonar, 1976; Hadfield 1978; Morse *et al.,* 1979; Coon *et al.,* 1984; Morse and Morse, 1984a; Morse, 1986). Chemosensory receptors controlling metamorphosis of *Haliotis rufescens* (marine pro-

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sobranch gastropod) larvae in response to exogenous γ-aminobutyric acid (GABA) and GABA-mimetic compounds purified from the naturally recruiting host algae recently have been directly labelled and characterized (Trapido-Rosenthal and Morse, submitted).

Signaling in many neuronal receptor systems has been found to involve depolarization of specialized cells in response to an appropriate stimulus (review by Aidley, 1978). We have suggested that the perception of inductive cues by larvae may rely on the stimulus-mediated depolarization of cells in a sensory/inductive pathway (Baloun and Morse, 1984). Consistent with this hypothesis, we previously observed that: (1) an increase in the concentration of K^+ in seawater is sufficient to induce settlement and metamorphosis of *Haliotis rufescens* larvae; (2) a decrease in external K^+ concentration inhibits the induction of metamorphosis in larvae of this species by GABA; and (3) sulfonyl isothiocyanostilbene, an anion channel blocker, also inhibits the larval response to GABA (Baloun and Morse, 1984). Since K^+ is capable of directly influencing cell membrane potential (Hodgkin and Horowicz, 1959), we further suggested that increasing external K^+ might be an effective technique for inducing metamorphosis in other marine invertebrate larvae. Evidence presented here demonstrates that increased potassium is effective as a morphogenetic inducer with larvae of several species of marine invertebrates. Larvae of the nudibranch *Phestilla sibogae* (Mollusca), the prosobranch *Astraea undosa* (Mollusca), and the polychaete *Phragmatopoma californica* (Annelida) respond to increased external K^+ in seawater with complete metamorphosis.

Materials and Methods

Larval culture

Larvae were obtained from eggs of adult specimens of *Phestilla sibogae* maintained at the Kewalo Marine Laboratory, University of Hawaii. Fertilized egg masses were collected within hours of deposition, and maintaining in flowing seawater at 26.6 ± 0.4°C until larvae were hatched by gentle agitation of the egg cases at 5–6 days post-fertilization. Hatched larvae then were transferred into seawater with 60 ppm penicillin G and 50 ppm streptomycin sulfate in chambers aerated by airlift recirculation, where they developed to a stage of competence to respond to inducers of metamorphosis by 8 days post-fertilization (Bonar and Hadfield, 1974; Hadfield, 1977). An inductive extract of the natural recruiting substrate (the coral *Porites compressa;* Hadfield, 1977) was prepared by soaking broken tips of *P. compressa* for 20 h in 0.56 *M* NaCl, buffered to pH 8.3 with 10 m*M* tris(hydroxymethyl)aminomethane. The aqueous extract was passed sequentially through paper, 1.2 and 0.22 μm Millipore®, and Amicon® (nominal 10,000 MW exclusion) filters, and then adjusted to MBL seawater composition (see below) by the addition of K, Ca, and Mg salts and water before final dilution. The larval response to the extract is dose-dependent. For the coral extract used in experiments reported here, a dilution of 1 part of the MBL-adjusted extract to 20 parts standard MBL seawater induced approximately 30% of the larvae to metamorphose within 24 h.

Larvae of *Haliotis rufescens* were obtained by controlled fertilization, with gametes spawned by gravid adults after a brief exposure to dilute hydrogen peroxide (Morse *et al.,* 1977). Larvae were cultured in flowing 5 μm-filtered, ultraviolet-irradiated seawater at 15.0 ± 1.0°C; these uniformly developed to a stage of competence to respond to inducers of metamorphosis by 7 days post-fertilization (Morse *et al.,* 1980a). The amino acid GABA (γ-aminobutyric acid) effectively mimicks the action of the natural

recruiting substrate (crustose red algae such as *Lithothamnium* and *Lithophyllum* spp.; Morse *et al.*, 1979, 1980a, b, c; Morse and Morse, 1984b). Conversely, the natural inducer purified from the recruiting algae mimicks the action of GABA at mammalian brain GABA receptors (Morse *et al.*, 1984; Morse, 1986). GABA at 4×10^{-7} *M* in MBL seawater provides a submaximal response that is sensitive to both facilitating and inhibitory conditions (Baloun and Morse, 1984).

Larvae of *Astraea undosa* were obtained by the mixing of gametes from adults stimulated to spawn by brief thermal shock in seawater at 24–25°C (an increase of 4–5°C above the broodstock ambient). These larvae were cultured at 18 ± 2°C in flowing 5 μm-filtered, ultraviolet-irradiated seawater. The natural inducer of metamorphosis for *A. undosa* larvae has not yet been precisely defined, although crustose red algae and GABA are effective (Markell and Morse, in prep.).

Larvae of *Phragmatopoma californica* were obtained by the mixing of gametes from adults stimulated to spawn by removal from their individual tubes (Jensen and Morse, 1984). Cultures of larvae, fed with the microalga *Dunaliella* spp., were maintained at 20 ± 1°C in stirred 5 μm-filtered, ultraviolet-irradiated seawater through development to metamorphic competence by 3 weeks post-fertilization. After development of metamorphic competence, the temperature of larval cultures was maintained at 17 ± 2°C. The natural recruiting substratum for this species is the anterior tube of the conspecific adult (Jensen and Morse, 1984). Clean preparations of inductive material were collected from tubes built in laboratory culture by adults provided with 0.45–0.50 mm glass beads (Glasperlen; B. Braun Melsungen AG Mfg., obtained from Van Waters and Rogers, Los Angeles, California; *cf.*, Jensen and Morse, 1984). Larvae of *H. rufescens*, *A. undosa*, and *P. californica* were cultured and used in experiments at the University of California, Santa Barbara.

Artificial seawater

Defined seawater medium based on the Woods Hole Marine Biological Laboratory (MBL) recipe (Cavanaugh, 1956) was used for all control assays, and was modified in experimental media by the addition of chloride salts to increase the concentrations of selected cations. The salt and ion concentrations of the MBL medium were summarized previously for reference (Baloun and Morse, 1984). The final pH of media ranged from 7.8 to 8.1 without adjustment. Prior to use, the antibiotics penicillin and streptomycin were added to the media, at concentrations of 50 and 60 ppm respectively for media used with *P. sibogae*, and at a concentration of 150 ppm each for media used with *H. rufescens* or *A. undosa*. No antibiotics were included in assays with *P. californica*.

Sigma and Mallinckrodt Chemical analytical reagent grade salts were used in the construction of artificial seawater media, with the exception of the highly hygroscopic salt $MgCl_2 \cdot H_2O$, which was purchased as a 4.9 *M* stock solution from Sigma Chemical Company. Tetraethylammonium chloride (TEA), a potassium blocker, was obtained from Eastman Kodak Company. Antibiotics were purchased from Sigma Chemical Company.

Assays of induction

The induction of metamorphosis of *P. sibogae* larvae was determined by the percentage of 11 or 12 day old competent larvae that discarded their shells and elongated into the characteristic juvenile form (Hadfield, 1978) after 1–3 days of exposure to a test medium. Duplicate or triplicate assays using 20 larvae in 20 or 25 ml of artificial

seawater medium were carried out in lidded 40 ml Stender dishes at approximately 25°C.

The induction of plantigrade attachment of *H. rufescens* and *A. undosa,* an accurate indicator of metamorphic commitment (Morse *et al.,* 1979, 1980b; Markell and Morse, in prep.), was quantitated as the percentage of larvae firmly attached by the foot, as a function of time of exposure. Approximately 200 to 300 competent larvae were tested in each glass vial (2.4 cm diam.) with 10 ml of artificial seawater medium; test vials of larvae were incubated in duplicate at 15.0 ± 1.0°C for *H. rufescens,* and at 18 ± 2°C for *A. undosa.* Completion of metamorphosis was verified by loss of the velar swimming organ and the initiation of adult shell growth.

The induction of metamorphosis of *P. californica* larvae was measured as the percentage of larvae that dropped provisional setae and rotated the anterior tentacles forward (Jensen and Morse, 1984). The induction of metamorphosis was assayed as a function of cumulative time of exposure for five larvae in each vial (2.4 cm diam.) with 10 ml of medium. Completion of metamorphosis was confirmed by the development of the caudal tail. Data for all species are presented as the mean percentage of larvae responding, determined from 2 to 4 replicates from single representative experiments with standard deviation (S.D.) as indicated.

Results

Larvae of three species of gastropod mollusc and one species of polychaete annelid metamorphosed in response to increased external K^+ in seawater, in the absence of any other source of inductive stimulation. The response, measured as percent attachment or metamorphosis, was dose-dependent and yielded an approximately bell-shaped curve of effect as a function of concentration. At the lower limit, the response declined as the concentration of K^+ approached 0 m*M* excess, the non-inductive MBL seawater condition. The upper limit of the response was imposed by the onset of paralytic effects of high doses of excess K^+. However, within the effective tolerated range, the larvae remained healthy and active, and when induced, underwent complete metamorphosis.

Larvae of *Phestilla sibogae* showed an optimal response to increased external K^+ continuously provided at 20 m*M* excess (Fig. 1). By 72 h exposure to this concentration, 90% of the larvae metamorphosed into elongated shell-less juveniles. Attachment behavior, preceding the actual discarding of the shell, was not an unambiguous indicator of induction for *Phestilla;* thus we used completion of metamorphosis rather than initiation of the response as the measured parameter.

Larvae of *H. rufescens* and *A. undosa* displayed a rapid behavioral change in the presence of inducer, which provided a useful index of the induction of metamorphosis, and allowed quantitation of the initiation of the response. The attachment response, followed by complete metamorphosis, was optimally induced in *H. rufescens* larvae by about 10 m*M* excess K^+ (Fig. 2). Similarly, attachment and subsequent metamorphosis of *A. undosa* larvae occurred optimally in response to approximately 10 m*M* K^+ excess (Fig. 3).

Larvae of *Phragmatopoma californica* also underwent complete metamorphosis in response to excess K^+ (Fig. 4). The larvae were responsive over a broad range of excess K^+ concentrations. The lowest concentration of excess K^+ of 5 m*M,* which was ineffective with the other species, produced an average of 75% metamorphosis by 24 h exposure. Increasing concentrations $\geq$20 m*M* excess K^+ produced progressive evidence of toxicity; after 5 days at these concentrations, post-metamorphic development of the caudal tail appeared stunted.

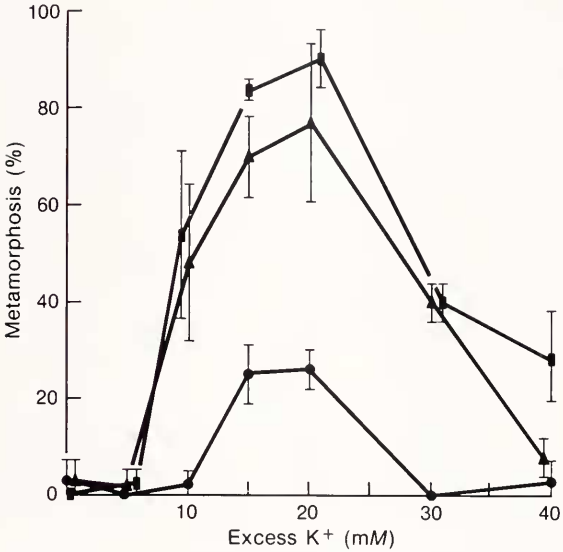

FIGURE 1. Induction of metamorphosis of larval *Phestilla sibogae* by increased external potassium. Potassium was added in excess to MBL seawater as KCl. The percentage of larvae metamorphosed was scored at 24 h (●), 48 h (▲), and 72 h (■) of continuous exposure. Data are averages of duplicates, with standard deviations indicated by vertical bars.

The effects of excess K^+ in combination with those of substratum-derived inducers or their analogs were additive. Since the response of larvae approached a maximum limit of 100% with increasing durations of exposure and higher concentrations of an

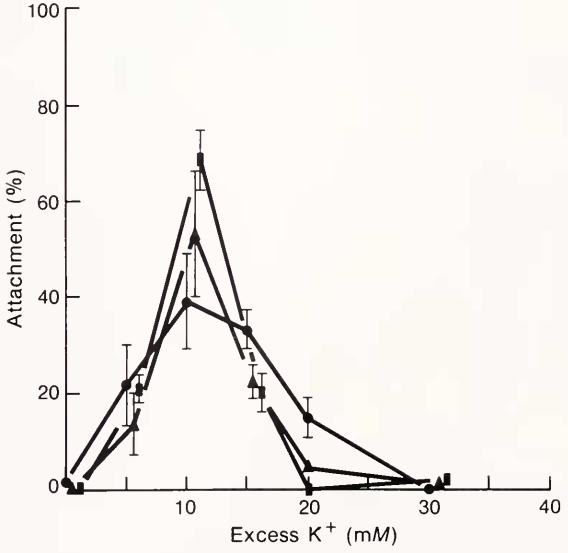

FIGURE 2. Induction of attachment of larval *Haliotis rufescens* by increased external potassium. The percentage of larvae attached was scored at 22 h (●), 48 h (▲), and 71 h (■) of continuous exposure, with other details as in the legend for Figure 1.

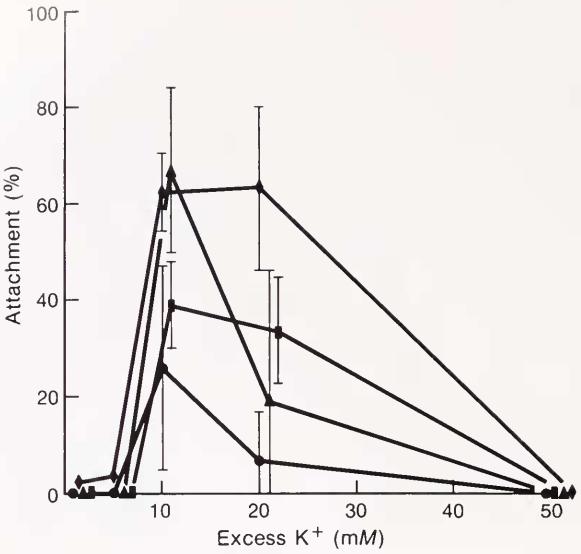

FIGURE 3. Induction of attachment of larval *Astraea undosa* by increased external potassium. The percentage of larvae attached was scored at 5 h (●), 22 h (▲), 49 h (■), and 92 h (◆) of continuous exposure, with other details as in the legend for Figure 1.

inducer, this additivity was most apparent at shorter exposure times and at lower concentrations. At 24 h, an excess of 10 m*M* K^+ nearly doubled the response of *P. sibogae* larvae to coral extract (Fig. 5). At longer durations of exposure (48 and 72 h),

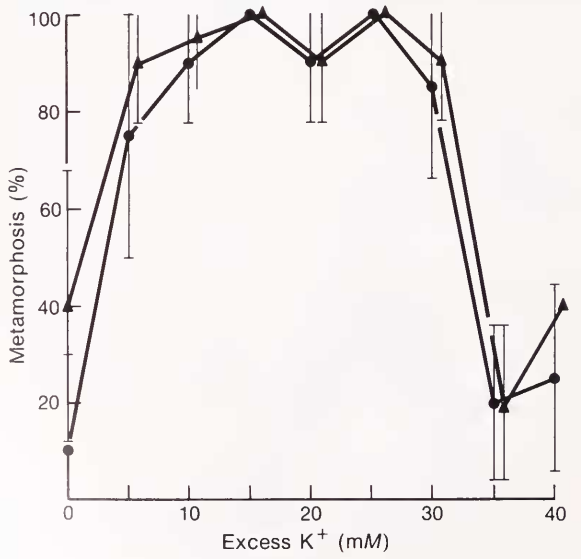

FIGURE 4. Induction of metamorphosis of larval *Phragmatopoma californica* by increased external potassium. The percentage of larvae metamorphosed was scored at 24 h (●), and 48 h (▲) of continuous exposure. Data are averages of four replicates, with standard deviations indicated by vertical bars. In parallel incubations, tube material produced by conspecific adults induced 60 (±28) % and 70 (±42) % of the larvae to metamorphose by 24 h and 48 h, respectively.

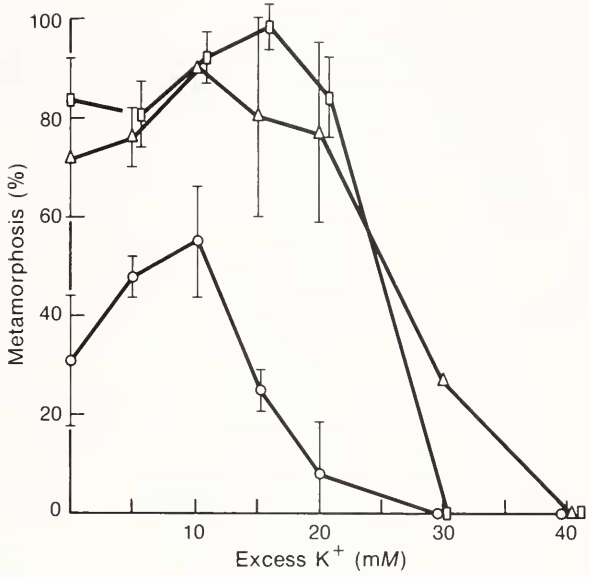

FIGURE 5. Induction of metamorphosis of larval *Phestilla sibogae* by excess potassium, when present in addition to inductive coral extract. The percentage of larvae metamorphosed was scored at 24 h (○), 48 h (△), and 72 h (□) of continuous exposure. The coral extract was prepared as described in the text, with other details as in the legend of Figure 1. For values obtained in the absence of coral extract, see Figure 1.

the percentage of larvae induced by coral extract to metamorphose was only slightly increased by excess K^+ (Fig. 5). The additive effect of K^+ and GABA as inducers of metamorphosis for *H. rufescens* was described previously (Baloun and Morse, 1984), and is presented here in more detail (Fig. 6). The presence of 5 and 10 m*M* excess K^+ enhanced the metamorphic response of the larvae at 22 and 48 h. The response was not greater than the sum of the responses to each inducer separately (compare Fig. 2), indicating that the additive effect of K^+ with GABA is not synergistic. No tests of additive effects with K^+ were performed with larvae of *A. undosa* or *P. californica*.

The inductive action of KCl, when added in excess to defined seawater medium, is specifically a function of the K^+ ion concentration, and is not dependent on an increase in osmotic pressure or paired anion concentration. Thus, comparable increases in the concentrations of other seawater cations have no inductive effect with larvae of *Haliotis rufescens* (Baloun and Morse, 1984). Competent larvae of *P. sibogae* in MBL seawater with excess NaCl (at 15, 30, or 60 m*M*), or with $CaCl_2$ or $MgCl_2$ (at 7.5, 15, or 30 m*M*), showed normal premetamorphic swimming behavior, and 0 (±0 S.D.) % of the larvae metamorphosed by 70 h exposure. Similarly, 0 (±0 S.D.) % of the larvae of *P. californica* metamorphosed in excess Na^+ or Mg^{2+} media (at the same concentrations as above) through 70 h; however, excess Ca^{2+} induced up to 50 (±14 S.D.) % metamorphosis by 48 h of exposure (data not shown).

Tetraethylammonium chloride (TEA), a quarternary cation known to block some K^+ channel currents (Armstrong, 1974; Hermann and Gorman, 1981) has been demonstrated to inhibit the metamorphic response of *H. rufescens* larvae to 12 m*M* excess K^+, at a concentration of 5×10^{-5} *M* TEA (Baloun and Morse, 1984). In contrast, the inductive action of excess K^+ with *P. sibogae* and *P. californica* larvae was insensitive to the presence of TEA, over a range of concentrations (Table IA, B). TEA did not inhibit the response of *H. rufescens* larvae to GABA (Baloun and Morse, 1984)

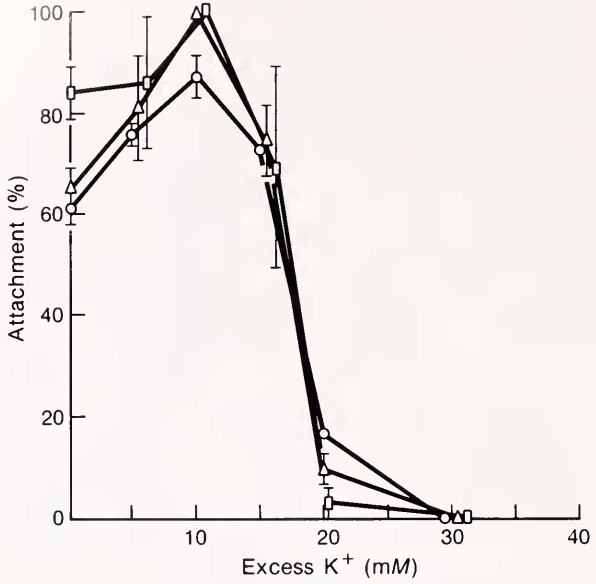

FIGURE 6. Induction of attachment of larval *Haliotis rufescens* by excess potassium, when present in addition to the chemical inducer GABA. The concentration of GABA was 4×10^{-7} *M*. The percentage of larvae attached was scored at 22 h (○), 48 h (△), and 71 h (□) of continuous exposure, with other details as in the legend for Figure 1. For values obtained in the absence of GABA, see Figure 2.

or the response of *P. sibogae* larvae to coral extract (Table IA). The effect of TEA on the response of *A. undosa* larvae has not yet been tested.

DISCUSSION

The gastropods *Phestilla sibogae, Haliotis rufescens, Astraea undosa,* and the polychaete *Phragmatopoma californica* differ in their native habitats. This difference is reflected in the dissimilar nature of the metamorphic signals recognized by the larvae. Larvae of *Phestilla,* a small subtropical carnivore, settle and metamorphose on the prey species *Porites compressa* in Hawaiian coral reefs (Hadfield, 1977). Larvae of the macroalgal herbivores *Haliotis* and *Astraea* metamorphose on substrates covered by various crustose red algae, on which the juveniles feed in the intertidal and subtidal zones of the southern California coast (Morse *et al.,* 1979; 1980a; and unpub. obs.). Larvae of the filter-feeding tubeworm *Phragmatopoma* metamorphose gregariously in response to the anterior tube material of conspecific adults (Jensen and Morse, 1984).

Certain neuroactive compounds applied externally have been demonstrated to induce larval metamorphosis in several species. These inducers are specific, and have been suggested to act either as chemical analogs of the natural substratum-derived inducers, or as precursors or active components within a signaling pathway. Choline (at 7.2×10^{-2} *M*, as choline chloride) induces metamorphosis of 60–85% of *P. sibogae* larvae (Hadfield, 1978; Hadfield *et al.,* in prep.), but has no inductive effect (at 10^{-3} *M*) on *H. rufescens* larvae (Morse *et al.,* 1979). GABA (at 10^{-6} to 10^{-3} *M*) induces virtually 100% of *H. rufescens* larvae to settle and attach, and (at 10^{-6} *M*) to complete metamorphosis (Morse *et al.,* 1979); GABA also induces settlement and metamorphosis of *Astraea undosa* larvae (Markell and Morse, in prep.) but has no inductive effect (at

TABLE I

Effect of the potassium channel blocker (TEA) on responses of larvae to excess potassium or the substrate-derived inducer

Species	Inducer[1]	Conc.[2]	TEA[3] (M)	Metamorphosis[4] (% ± S.D.)
A. *Phestilla sibogae*	None	—	0	15 ± 3
			5×10^{-6}	3 ± 3
			5×10^{-5}	1 ± 1
			5×10^{-4}	0 ± 0
	Excess K^+	20	0	71 ± 5
			5×10^{-6}	65 ± 25
			5×10^{-5}	70 ± 16
			5×10^{-4}	65 ± 17
	Coral extract	1:15	0	100 ± 0
			5×10^{-6}	94 ± 6
			5×10^{-5}	96 ± 3
			5×10^{-4}	93 ± 8
B. *Phragmatopoma californica*	None	—	0	0 ± 0
			5×10^{-6}	0 ± 0
			5×10^{-5}	0 ± 0
			5×10^{-4}	0 ± 0
	Excess K^+	15	0	80 ± 0
			5×10^{-6}	80 ± 0
			5×10^{-5}	80 ± 28
			5×10^{-4}	90 ± 14
	Conspecific tube material	—	0	80 ± 0

[1] All tests in MBL seawater. Excess K^+ added as KCl; extract prepared from coral as described in text.
[2] Concentration of excess K^+ in mM; coral extract diluted 1 part to 15 parts MBL seawater.
[3] TEA (tetraethylammonium chloride).
[4] Larval metamorphosis at 48 h, averaged from triplicate samples for *P. sibogae*, and duplicate samples for *P. californica*.

9.7×10^{-3} or 9.7×10^{-2} M) on *P. sibogae* larvae (Hadfield, 1984). Certain catecholamines show different activities as inducers of metamorphosis in larval *P. californica* (Jensen and Morse, unpub. obs.), and cause partial metamorphosis of larval *P. sibogae* (Hadfield, 1984), but have no inductive effect on *H. rufescens* (at 10^{-3} M; Morse *et al.*, 1979) or on *A. undosa* (Markell and Morse, in prep.). Catecholamines also have been shown to induce metamorphosis of the sand dollar *Dendraster excentricus* (Burke, 1983a), and the oyster *Crassostrea gigas* (Coon *et al.*, 1984).

In sum, the external chemical stimuli capable of inducing larvae of *Phestilla, Haliotis, Phragmatopoma,* and *Astraea* to metamorphose are restricted in activity by species-specificity, with the notable exception of potassium. As reported here, larvae of four species from two invertebrate phyla share a sensitivity to K^+ as an inducer of metamorphosis. Excess K^+ also has been found to induce metamorphosis of the urchin, *Lytechinus variegatus* (Cameron and Tosteson, in prep.). The inductive effect of external cations on larval metamorphosis was demonstrated previously by Spindler and Müller (1972) and Müller and Buchal (1973), who showed that planula larvae of *Hydractinia echinata* (Cnidaria) metamorphosed in response to increased K^+, Li^+,

Rb^+, or Cs^+. With the protocol used by Müller and colleagues (involving short exposures of larvae to high doses), K^+ proved to be the least effective inducer among the cations tested. Nonetheless, their result appears to support the generality of the effect of potassium, by extending the examples of responsive larvae to include species from four invertebrate phyla.

Since membrane potential in excitable cells is influenced by changes in the K^+ electrochemical gradient (Hodgkin and Horowicz, 1959; Hagiwara *et al.,* 1961; Monticelli, 1979; Schlue and Deitmer, 1984), we have suggested that increased external K^+ may activate metamorphosis by depolarizing externally accessible cells in an inductive pathway (Baloun and Morse, 1984). The magnitude of the depolarization resulting from the doubling of external K^+ concentration (generally found optimal in our experiments), as estimated from the Nernst equation, is on the order of $^+17$ mV. This putative depolarization appears to bypass the species-specific receptor-stimulus interaction, as verified both by pharmacological analyses that resolve the two effects in *Haliotis rufescens* larvae (Baloun and Morse, 1984), and by recent experiments directly characterizing the down-regulation of the chemosensory receptors controlling metamorphosis in this species (Trapido-Rosenthal and Morse, submitted).

Data reviewed previously indicate that both the chemosensory receptors and the potassium-depolarizable cell membranes that control settlement and metamorphosis of *H. rufescens* are located on the epithelial surface of the larvae (Baloun and Morse, 1984; Morse, 1986). These larvae also are found to be unaffected by tetrodotoxin, suggesting that the epithelia are not leaky (Baloun and Morse, 1984). In the experiments reported here, the technique involving total immersion of larvae in modified seawater media prevents a localized application of the potassium stimulus. It is possible, then, that excess K^+ might influence a variety of cells exposed on the surface of the larva, and induces metamorphic changes independently in different target tissues. However, the complex metamorphic changes in behavior, loss of larval specializations, and new growth of adult structures follow the same critical sequence whether induced by excess K^+ or by the natural substratum. Thus, we believe it more likely that depolarization with K^+ activates the normal morphogenetic pathway, which in turn coordinates the genetically programmed sequence of behavioral and developmental changes resulting in metamorphosis. The ability of excess K^+ to effect a rapid change in behavior from the swimming premetamorphic phase to the crawling juvenile phase, without affecting the behavioral responses of larvae to light and mechanical stimuli, also argues for a restricted effect of K^+ on the larvae.

The potassium channel blocker, TEA, inhibits the induction of metamorphosis by excess K^+ in larval *H. rufescens* (Baloun and Morse, 1984). The insensitivity of *P. sibogae* and *P. californica* larvae to TEA (Table IA, B) indicates that K^+ may act through channels different from those suggested to be involved in the potassium-induced metamorphosis of *H. rufescens* larvae (Baloun and Morse, 1984). Several classes of physically distinct channels that conduct K^+ currents in molluscan neurons have been identified in studies of their current kinetics, gating dependence, pharmacological sensitivity, and selectivity (Thompson, 1977; Westerfield and Lux, 1982; review by Edwards, 1982). Pharmacological analyses have shown that channels conducting potassium currents include those which are TEA-insensitive (Kostyuk *et al.,* 1980; Edgington and Stuart, 1981), blocked by internally applied TEA (Armstrong and Binstock, 1965), or sensitive to both internal and external TEA (Hermann and Gorman, 1979; 1981). Thus it appears possible that potassium channels mediate the induction of metamorphosis by K^+ in *P. sibogae* and *P. californica,* but that K^+ conductance through these channels is insensitive to external TEA.

The effectiveness of potassium does not imply that K^+ currents necessarily are

involved in transducing natural signals for metamorphosis. In fact, the activation of larval receptor cells by depolarization could be accomplished by cation influx or anion efflux involving any of the physiologically relevant ions. For example, the response of *H. rufescens* larvae to GABA may depend in part on a depolarizing efflux of chloride (Baloun and Morse, 1984). In addition, the observation made here that *P. californica* larvae metamorphose in excess Ca^{2+} medium may suggest a role for calcium in signal transduction in these larvae. Calcium fluxes also have been implicated in the modulation or transduction of the morphogenetic signal in *H. rufescens* larvae (Morse *et al.*, 1980a; Baloun and Morse, 1984). Thus, while the common requirement of signal transduction following receptor activation may be depolarization, the mechanisms driving and responding to this event may vary among diverse species.

The results presented here demonstrate the effectiveness of potassium as a morphogenetic inducer in larvae of four marine invertebrate species. Subsequent electrophysiological and biochemical studies employing this effect should prove useful to further characterize the molecular, cellular, and neuronal mechanisms involved in substratum recognition, and the control of larval settlement and metamorphosis.

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